Radial Propagation of Muscle Action Potential along the Tubular System Examined by Potential-sensitive Dyes

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ABSTRACT Isolated single (Xenopus) muscle fibers were stained with a non-permeant potential-probing dye, merocyanine rhodanine (WW375) or merocyanine oxazolone (NK2367). When the fiber was massively stimulated, an absorption change (wave a), which seemed to reflect the action potential, occurred. Simultaneous recording of optical changes and intracellular action potentials revealed that the time-course of wave a was slower than the action potential: the peak of wave a was attained at 1 ms, and the peak of action potential was reached at 0.5 ms after the stimulation. This difference suggests that wave a represents the potential changes of the whole tubular membrane and the surface membrane, whereas the action potential represents a surface potential change. This idea was substantiated by recording absorption signals preferentially from the surface membrane by recording the absorption changes at the edge of the fiber. Wave a obtained by this method was as quick as the intracellular action potential. The value of radial conduction velocity of action potential along the T system, calculated by comparing the action potential with wave a, was 6.4 cm/s at 24.5°C, in fair agreement with González-Serratos (1971. J. Physiol. [Lond.]. 212:777-799). The shape of wave a suggests the existence of an access delay (a conduction delay at the orifice of the T system) of 130 μs.

INTRODUCTION

In the preceding paper (Nakajima and Gilai, 1980) we have described absorption signals of single (Xenopus) muscle fibers stained with potential-sensitive dyes. We used the nonpermeant dyes, merocyanine rhodanine (WW375) and merocyanine oxazolone (NK2367). Upon stimulating the fiber, we observed three optical changes, which we called wave a, wave b, and wave c. Only wave a seemed to reflect the membrane potential change, and thus we can call wave a the optical action potential.

The optical action potential starts almost immediately after massive stimulation and reaches the peak at about 1 ms. This time-course is slower than that of the intracellularly recorded electrical action potential. In the present paper we shall examine this difference in time-course between the electrical and the optical action potentials. The main purpose is to estimate the radial
conduction velocity through the T system. Preliminary accounts have appeared (Nakajima and Gilai, 1977 and 1978). Some of the results were presented at the Symposium on Excitation-Contraction Coupling held at the University of California, Los Angeles (1979; proceedings in preparation).

METHODS

The materials and the methods were the same as described in the preceding paper (Nakajima and Gilai, 1980). For optical analysis we used almost exclusively the signals at 702 nm in WW375-stained fibers: at this wavelength the magnitude of wave a is maximum (Nakajima and Gilai, 1980).

RESULTS

Massive Stimulation

Under natural conditions the muscle action potential propagates along the muscle fiber surface. This surface action potential, in turn, evokes an action potential in the transverse tubular system (T system), and it travels radially toward the fiber axis (Huxley and Taylor, 1958; Costantin, 1970; Costantin and Taylor, 1971; Bastian and Nakajima, 1974). In experiments in which the muscle fiber is subjected to the brief, massive stimulation that we have used routinely, we expected that the surface action potential would be synchronously evoked all along the fiber, and that this surface action potential would, in turn, trigger the excitation of the T system. We did not expect that the massive stimulus itself would directly excite the T system, because the diameter of the tubules is very small and because of this the threshold is high (Erlanger and Gasser, 1937). The results shown in Fig. 1 agree with these expectations.

Record A of Fig. 1 illustrates the typical sequence of light transmission changes that occur upon a brief (0.05-ms), massive stimulation of a fiber stained with merocyanine rhodanine (WW375), whereas record B shows transmission changes when the same fiber is stimulated through the "conductive mode" (the fiber was stimulated at a location away from the region where the optical signals were obtained). The optical signs illustrated in both A and B were obtained with a wavelength of 702 nm from an area 300-µm long and the whole width of the fiber.

As seen from record A of Fig. 1, the massive stimulus (arrow) almost immediately evoked an abrupt decrease in light transmission (wave a), and this was followed by waves b and c. In the preceding paper we concluded that only wave a represents the action potential of muscle membrane, whereas waves b and c are light-scattering changes concomitant with muscle movement. The main evidence for this conclusion is that wave a shows up only after the staining of the muscle fiber, and that the direction and magnitude of wave a are markedly dependent on the light wavelength.

In record B of Fig. 1, which was obtained by stimulating the fiber in a conducted mode, wave a occurred after a long latency (which represents the conduction time from the stimulating point) followed by waves b and c. In record B the optical signal appears to be more rounded than in record A. This rounding of the wave shape occurs partly because of conduction of action
potential from one end of the slit to the other (the region under the slit is not synchronously excited) and partly because the conducting action potential itself has an exponentially rising foot.

In the experiment shown in Fig. 1 C, the slit was made very short (100 µm, referred to the actual dimension of the fiber), so that this short region would be almost simultaneously excited, even with the conductive mode of stimulation. In this case wave a evoked by the conducted mode would be almost the same as wave a evoked by the massive stimulation. Curve 1 of Fig. 1 C was obtained when the fiber was stimulated in the conducted mode, whereas curves 2 and 3 were all obtained with the massive stimulation of 0.05 ms duration: the stimulus current intensity was twice the threshold in curve 2.

**Figure 1.** Dye-absorption signals produced by different modes of stimulus in a single muscle fiber stained with WW375 (100 µg/ml) for ~15 min. Wavelength, 702 nm. Averaged 16 times. Calibrations refer to ΔI/I. Transmission increase, upward direction. Arrows indicate the beginning of the stimulus. In records A and B, the slit size = (fiber width) × 300 µm, referred to the actual dimension of the fiber. (A) Massive stimulation, stimulus intensity 2 times the threshold. (B) Stimulated in the conducted mode, namely, the stimulus was delivered through a platinum wire insulated except for the tip (cathode) located away from the slit area. (C) The slit size = (fiber width) × 100 µm. C(1), stimulated in the conductive mode. C(2), stimulated massively; stimulus intensity 2 times threshold. C(3), stimulated massively; stimulus intensity 4 times the threshold. The height of wave a slowly declines from A, B and then from C(1) to C(3): this decline is due to the spontaneous fading of the signal. The double arrow indicates a possible incipient wave b. Fiber diameter, 110 µm. Sarcomere length, 2.6 µm. Room temperature, 25°C. Records A and B are photographs from an oscilloscope connected to an averager. C records are tracings from the photographs.
and four times threshold in curve 3. These records show that the time-courses of all wave a's are much the same, irrespective of the mode of stimulation. The falling phase of curve 1 is slightly distorted (double arrow), suggesting the beginning of latency relaxation occurring at a place away from the slit location. The size of wave a is largest in curve 1 and smallest in curve 3: this merely reflects the fading effect discussed in the preceding paper (Nakajima and Gilai, 1980).

Since the signal for massive stimulation with an intensity up to four times threshold is not faster than the signal for a propagated action potential, massive stimulation does not seem to excite the T system directly. Instead, the massive stimulation first synchronously excites the surface membrane, and the surface action potential, in turn, evokes the T system action potential, which radially conducts into the fiber axis. This justifies our use of massive stimulation for the analysis of radial conduction.

Comparison of Optical and Electrical Action Potentials

Fig. 2 shows an example of simultaneous recording of optical and electrical signals from a fiber stained with WW375. The optical signal was recorded
from a region 500 \( \mu \text{m} \) long and the whole width of the fiber. The electrical signal was obtained through an intracellular microelectrode inserted near the end of this region. Record A is a photograph of an action potential and optical signal simultaneously recorded on an oscilloscope. In this record the optical signal was taken at 702 nm and was photographed with the downward direction corresponding to transmission increase (this direction is opposite to the convention adopted for other figures of this series of papers). In this particular fiber we were able to record four consecutive action potentials without the electrode being dislodged. Thus, we obtained, as shown in record B, the average of the four optical signals (averaged with an averager) and the

### Table I

<table>
<thead>
<tr>
<th>Fiber reference</th>
<th>Diameter ( \mu \text{m} )</th>
<th>Height ( \text{m} )</th>
<th>Time to peak ( \text{ms} )</th>
<th>( \Delta I / I ) ( \times 10^{-4} )</th>
<th>Time to peak ( \text{ms} )</th>
<th>Access delay ( \mu \text{s} )</th>
<th>( \Theta ) ( \text{cm/s} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>MF-111</td>
<td>108</td>
<td>135</td>
<td>0.48</td>
<td>6.7</td>
<td>1.00</td>
<td>133</td>
<td>5.8</td>
</tr>
<tr>
<td>MF-112</td>
<td>114</td>
<td>113</td>
<td>0.86</td>
<td>6.4</td>
<td>1.33</td>
<td>266</td>
<td>8.5</td>
</tr>
<tr>
<td>MF-113</td>
<td>114</td>
<td>145</td>
<td>0.47</td>
<td>7.1</td>
<td>0.80</td>
<td>100</td>
<td>10.7</td>
</tr>
<tr>
<td>MF-113</td>
<td>102</td>
<td>123</td>
<td>0.43</td>
<td>4.9</td>
<td>0.98</td>
<td>133</td>
<td>5.5</td>
</tr>
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<td>MF-113</td>
<td>108</td>
<td>143</td>
<td>0.28</td>
<td>6.6</td>
<td>1.00</td>
<td>133</td>
<td>5.1</td>
</tr>
<tr>
<td>MF-113</td>
<td>134</td>
<td>126</td>
<td>0.63</td>
<td>3.4</td>
<td>1.30</td>
<td>33</td>
<td>2.8</td>
</tr>
<tr>
<td>Mean</td>
<td>113</td>
<td>131</td>
<td>0.53</td>
<td>5.9</td>
<td>1.07</td>
<td>133</td>
<td>6.4</td>
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</table>

**Time to peak** refers to the time between the start of the massive stimulation and the peak of the waves. Stained with WW375 (100 \( \mu \text{g/ml} \)) for ~15 min. The optical signals were measured at 702 nm. Slit size = (fiber width) \( \times 300 \mu \text{m} \). \( \Theta \) is the conduction velocity in the T system. Sarcomere length was 2.5-2.8 \( \mu \text{m} \). Room temperature was 24-25\(^\circ\text{C}\). In MF-111 and 112, four responses were averaged. In MF-113-MF-128, the calculation was based only on the response to the first stimulus. In MF-113 the photographic contrast of the response to the first stimulus was bad: the calculation was carried out from a trace of this photograph made by a drafts person. The second response produced a good photograph, but the action potential was small. The same value of \( \Theta \) was obtained from the second response.

average of the four electrical action potentials (averaged from the photographs of four action potentials). As shown in Fig. 2, the time-course of the optical signal (wave a) was slower than that of the electrical action potential. Results of simultaneous optical and electrical recordings from six cells are summarized in Table I, which shows that the electrical action potential reaches its peak at 0.53 ms, and optical action potential at 1.07 ms, after the start of stimulus: the latter value is close to the data (0.93 ms) from the 18-cell sample of Table II of the previous paper (Nakajima and Gilai, 1980).

This lagging of the optical signal contrasts sharply with the findings of Ross et al. (1977), who obtained an exact coincidence of optical and electrical changes in the giant axon. As mentioned in the previous paper (Nakajima and Gilai, 1980), the time-courses of electrical and optical signals in muscle are probably different because the muscle action potential conducts radially
from the surface into the T system. The electrically measured action potential represents potential changes in the surface membrane (Falk and Fatt, 1964; Adrian et al., 1969; Nakajima et al., 1975), whereas the optical action potential should be proportional to the average of the potential changes of all the membranes including the T system and the surface membranes. The results presented in the following section support this idea.

Figure 3. Comparison of wave a's recorded either from edge or center of a fiber stained with WW375 (100 µg/ml) for ~15 min. Filled circles, wave a recorded from the edges of fiber (two records from both edges were averaged). Continuous step lines; wave a recorded from central part of the fiber. Photographs from an averager were traced and the heights were adjusted. Slit size = 11 µm x 500 µm. Wavelength, 702 nm. Averaged 64 times. Calibrations refer to ΔI/I. Transmission increase, upward direction. Arrows, the beginning of the stimulus. Fiber diameter, 111 µm. Sarcomere length, 2.7 µm. Room temperature, 24.5°C.

Edge-Center Experiments

In view of the results from the squid giant axon, we would expect that, in muscle, if we could obtain the optical signal from the surface membrane only, its time-course would coincide with the electrical signal. Performing such an experiment, however, is very difficult. We were able to obtain nearly the same optical signals by recording them from the edge of fiber.

As explained in the previous paper (Nakajima and Gilai, 1980), by appropriately positioning the X-Y diaphragm, we were able to get the optical signal coming predominantly from either the edge or from the central part of the fiber. When the optical signals are obtained from the edge of the fiber, they reflect the events occurring in the surface membrane and the peripheral part of the T system. In Fig. 3 we show a tracing of wave a's obtained from the edge of a fiber (filled circles), superimposed with a tracing of wave a obtained from the center of the fiber (stepwise lines). The heights of the two traces were
adjusted to make them approximately the same. As expected, the time-course of wave a recorded from the edge was faster than that obtained from the central part of the fiber. The result from four fibers (Table II) shows that wave a recorded from the edge, reaching its peak at 0.58 ms, is as fast as the electrically recorded action potential (0.53 ms, Table I). The conclusion that can be drawn from these experiments is that the slowness of wave a, compared with the electrical action potential, is caused by the conduction time of the action potential radially propagating from the surface into the central part of the fiber. If there were no T system in muscle, the time-course of the optical signal would correspond to that of the electrical action potential.

### Table II

<table>
<thead>
<tr>
<th>Dye</th>
<th>Fiber reference</th>
<th>Diameter</th>
<th>Slit width</th>
<th>ΔI/1</th>
<th>Time to peak</th>
<th>ΔI/1</th>
<th>Time to peak</th>
<th>Θ</th>
</tr>
</thead>
<tbody>
<tr>
<td>WW375</td>
<td>MF-82</td>
<td>145</td>
<td>15</td>
<td>0.6</td>
<td>0.42</td>
<td>1.7</td>
<td>0.89</td>
<td>8.6</td>
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<tr>
<td></td>
<td>MF-86</td>
<td>126</td>
<td>15</td>
<td>2.6</td>
<td>0.36</td>
<td>3.8</td>
<td>0.86</td>
<td>11.7</td>
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<tr>
<td></td>
<td>MF-87</td>
<td>135</td>
<td>12</td>
<td>2.9</td>
<td>0.72</td>
<td>3.8</td>
<td>1.10</td>
<td>9.1</td>
</tr>
<tr>
<td></td>
<td>MF-103</td>
<td>111</td>
<td>11</td>
<td>4.0</td>
<td>0.63</td>
<td>6.0</td>
<td>0.88</td>
<td>9.0</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>129</td>
<td>13</td>
<td>2.5</td>
<td>0.58</td>
<td>3.8</td>
<td>0.93</td>
<td>9.6</td>
</tr>
<tr>
<td>NK2367</td>
<td>MF-97</td>
<td>89</td>
<td>17</td>
<td>2.4</td>
<td>0.71</td>
<td>4.0</td>
<td>1.04</td>
<td></td>
</tr>
</tbody>
</table>

*Time to peak* was measured from the start of the massive stimulation. Stained with the dyes at 100 μg/ml for ~15 min. The signals were measured at 702 nm in WW375 experiments and at 680 nm in NK2367 experiments. Averaged 64 times. Slit width refers to the dimension of actual fiber; the slit length was 500 μm. Conduction velocity (Θ) was not calculated in the NK2367 experiment because the slit width was too large. Sarcomere length was 2.5–2.7 μm. Room temperature was 24.5–27°C.

**Radial Conduction Velocity—Simultaneous Recording**

The simultaneous recording of optical and electrical action potentials, such as that shown in Fig. 2, allowed us to determine the radial conduction velocity of action potential in the T system. The assumptions on which our calculation was based are as follows: (a) The slowness of the optical signal, in comparison with the electrical signal, results only from radial conduction through the T system (this is a reasonable assumption fairly well established by the experiments reported in the previous section). (b) The T system action potential has the same shape as the surface action potential (this is not proven and potentially misleading; see Discussion). (c) The radial conduction velocity is constant (this is not a serious assumption; see Discussion).

On the basis of these assumptions, the optical signals, which can be obtained experimentally, were reconstructed from the wave form of the surface action potential as follows: The fiber membrane is divided into the surface membrane and 20 parts of T system, each representing the tubular membrane included.
inside each of 20 concentric rings of equal width (the innermost ring is a core disk). First, the surface membrane, of course, produces the same action potential as that recorded intracellularly. Second, the tubular membrane included in the first ring (the outermost ring) produces an action potential of the same shape as the surface potential but with a delay calculated according to an arbitrarily chosen radial conduction velocity $\Theta$. Third, the tubular membrane included in the next ring produces a potential with one more delay. This procedure is repeated for the remaining concentric rings. Then, the height of the action potential of each part is made proportional to its membrane area (Mobley and Eisenberg [1975]; cf. Peachey [1965]). Next the total summation of potential changes in the 21 parts is calculated; this should correspond to the optical signal, provided that the chosen value of the velocity $\Theta$ is correct. These calculations are repeated with different values of $\Theta$, and the optimal value of $\Theta$ is searched so that the calculated wave form best fits, in the least-square sense, the observed optical signal.1

In Fig. 4 A, the series of continuous curves are the calculated optical signals with various values of $\Theta$. (The bin interval of $\Theta$ was twice as dense as shown here.) The points in Fig. 4 A represent the wave a recorded experimentally. In this case the best fit was obtained at $\Theta = 3.7$ cm/s. But none of the calculated curves seems to be a very good fit. There appears to be a delay in the rising phase of the observed optical signal compared with the calculated curves. This deficiency was corrected by introducing an “access delay,” a conduction delay between the surface membrane and the first ring of the T system. We have introduced an access delay with a 33.3-μs bin interval and searched for the value of access delay and the value of conduction velocity that best fit the experimental data. In Fig. 4 B, the curve with the best-fit values is illustrated. It is obviously an improvement over the curves illustrated in A.

Data from the successful experiments are summarized in Table I. (The criteria for success are described in the preceding paper [Nakajima and Gilai, 1980].) In only two cells (MF-111 and MF-112) of the six listed in Table I were we able to obtain records of four successive action potentials, and in each of the remaining four cells, we were able record a large action potential only once. It can be seen that the access delay is consistently found in all the fibers, with an average value of 133 μs. Radial conduction velocity along the tubular membrane ($\Theta$) becomes on the average 6.4 cm/s.

Similar calculations were performed with the results of merocyanine 540 experiments. In this case no attempt was made to introduce the access delay. The conduction velocity becomes 6.0 ± 1.2 cm/s (mean ± SEM, $n = 5$). The height of the action potential in this sample was 123 mV.

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1 The calculation assumes that each of the 21 membrane parts is illuminated with the same intensity of light. However, because the resting fiber stained with the dye absorbs and scatters the light, with the apparent absorption coefficient $\alpha$ given in Table I of the previous paper, each of the 21 parts is illuminated by a slightly different intensity of light. We introduced a correction factor for this effect into the program. The computation has revealed that even when the value of $\alpha$ was set at an unusually high value (115 cm$^{-1}$, corresponding to an absorption length constant of 87 μm), the calculated wave form of the optical signal was only negligibly different from the signal obtained assuming that there was no resting light absorption.
Conduction Velocity from Edge-Center Experiments

As explained in regard to Fig. 3, wave a recorded from the fiber edge is fast and the time-course is comparable to the surface action potential, whereas wave a picked up from the center part of the fiber is slow. These data were also used to estimate the radial conduction velocity. The procedure for this calculation is similar to the one explained above. The main difference is that, when the narrow slit is located at the fiber center, and a narrow band of light coming through the center part of the fiber is measured, all parts of the tubular membrane (at the periphery as well as near the axis) contribute to the absorption signal with equal weight. The results of the calculation are listed in Table I. In the calculation no access resistance was assumed. The value obtained (9.6 cm/s) is somewhat higher than that described above.

**Figure 4.** Estimation of radial conduction velocity of action potential by analysis of simultaneously recorded optical and electrical signals. Continuous lines are predicted optical signals calculated from the experimentally determined electrical signal (action potential). Each curve was calculated on the basis of a different radial conduction velocity. The filled circles are the optical signal actually recorded. Normalizing factors were introduced to each continuous curve to make the height approximately the same as the optical signal. (A) Zero access resistance assumed. (B) The best-fit curve among various assumed values of access delay and radial conduction velocity. The data are from the experiment illustrated in Fig. 2.

DISCUSSION

González-Serratos (1971) analyzed the speed of the contraction wave propagating radially in isolated single muscle fibers of *Rana temporaria* with the
cinematographic microscope. He obtained a radial conduction velocity of 7 cm/s at 20°C. Because we know that the T system is excitable, this conduction velocity corresponds to the action potential velocity along the T system, provided that the speed of excitation-contraction coupling and the speed of contraction are the same in all myofibrils located at various depths from the surface.

The present experiments on *Xenopus* have shown that the radial conduction velocity is 6.4 cm/s at 24.5°C (Table I). This value is roughly the same as (or somewhat less than) that obtained by González-Serratos (1971). In our calculation we assumed that the conduction velocity along the T system is constant. The optical measurement of the whole-fiber thickness is determined mainly by the potential changes in the surface membrane and the peripheral part of the T system, and thus, the result in Table I should be regarded as the conduction velocity of the T system located relatively near the surface. It could be that the velocity near the axis is faster than 6.4 cm/s (as suggested by the calculation of Adrian and Peachey, 1973). The fact that the velocity obtained from the edge-center experiments was slightly larger (9.6 cm/s) at least agrees with the notion that the velocity near the axis is faster. But because the signal-to-noise ratio in the edge-center experiments is small, the value obtained from the edge-center experiments would be less reliable than those obtained by comparing the electrical and optical action potentials.

In our calculation we assumed that the shape of the action potential in the T system is the same as that of the surface action potential. This assumption is arbitrary and might produce a serious error. However, the fact that our value roughly agrees with that of González-Serratos indicates that the simplest explanation for all the results is that the various assumptions on which González-Serratos's conclusion and our analysis were based are approximately correct.

The existence of an access delay of ~130 μs appears to indicate the existence of access resistance, as proposed by Adrian and Peachey (1973). But the access resistance predicted from our result could be less than that of Adrian and Peachey (1973). According to Adrian and Peachey's (1973) simulation on *Rana temporaria* muscle, when an access resistance of 150 Ω cm² (corresponding to an access delay of ~300–500 μs) is introduced, the T system action potential at the very peripheral part becomes small and prolonged and the radial conduction velocity becomes slow (θ = 2.5 cm/s for the peripheral part—20 μm from the surface—of the T system; measured from Fig. 2 of Adrian and Peachey [1973]). Adrian and Peachey (1973) further showed that when no access resistance is introduced (this does not necessarily mean no access delay), the action potential of the T system has much the same shape as the surface action potential, and the conduction velocity becomes faster (4.3 cm/s for the periphery of the T system). On balance, this latter model appears to be somewhat more consistent with the results of our optical experiments. This question, as well as many others relating to the properties of action potential in T system, could be answered by detailed simulation, such as Adrian and Peachey (1973) have done, based on newly published experimental data.
When comparing González-Serratos's (1971) experiments with our present experiments, we feel that if the sole purpose is to obtain the value of the radial velocity, his method is simpler and more elegant than ours. The significance of our experiments lies in the fact that we have obtained González-Serratos's value by a wholly different method based on different assumptions. Also, the present study has extended the usefulness of the potential-sensitive dye technique to the analysis of the events taking place in the intracellular membrane system.

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